Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 3 [Special Issue V] 2014: 243-246

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

The study of Sodium silicate effects on the total protein content, and the activities of catalase, Peroxidase and Superoxide Dismutase of *Vicia faba* L.

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ABSTRACT
Silica is the second common element of soil content which has positive effects on the resistance of plants against biotic and abiotic stresses. This element can increase the yield, decrease the evaporation and perspirationand moreover, causes increasing of production of antioxidant enzymes , and less sensitivity to some fungal diseases. In the present study, the effects of silica on the total protein content and activity of some antioxidant enzymes in vicia faba L. were studied. The seeds of plant were treated by 0 (as control), 1.5 and 3mM of Sodium silicate. There were three repeats for all treatments. The result showed that 1.5mM treatment significantly increased the total protein content in comparison to control samples.The activity of Catalase in the 3mM treatment of Sodium silicate was significantly increased. The activity of Peroxsidase in the 3 mM treatments of Sodium silicate was significantly increased. In 3mM treatments of Sodium silicate also increased the activity of Superoxide Dismutase. Based on the results, it can be concluded that Nano silica particles can increase the activity of some antioxidant enzymes in broad bean, which in turn, brings about less damages caused by reactive oxygen species, and protects the plant's physiological processes against stresses.
Key words: *Antioxidant Enzymes, Total protein , Sodium silicate , Vicia faba L.*

Received 12.05.2014 Revised 15.07.2014 Accepted 15.08. 2014

INTRODUCTION

*Vicia faba*L. is one of the *Fabaceae.* This plant is annual grass, with 80-100 cm height,. The flowers of broad bean are white with black or purple spots. The seeds are sheathed and the fruits, seeds and flowers have medical usages. *Vicia faba* L. is hetero fertilized with 2n=12. Because of possessing of high percentage of proteins (30-34%) , this plant is alimentary- worth. Environmental stress causes reduction of balance between reactive oxygen species and antioxidant defense of plants (3). Superoxide Dismutase as one of the metaloproteins, can catalyse $2O_2^{0-} \rightarrow O_2 + O_2^{0-}$[12]. SOD indeed, produces H_2O_2. (7). Catalase (CAT) is one of the H_2O_2 scavenger which can catalyze the reaction of $2H_2O_2 \rightarrow 2H_2O + O2$ as a metal protein [9]. After an smooth increasing of catalase activity which is associated with shortage of water in root and leaves, this activity in leaves would be stable at constant level, and is reduced in heavy shortage of water in root, that may bring about inactivation of catalase [8]. After oxygen, silica is the second structural element in the earth which is non mobile in the plants. Although silica is not necessary for plants, higher plants need it to have optimum growth [19, 6]. The most effect of silica on plants is related to the resistance against biotic and abiotic stress [16, 15]. As the cell wall of plants prevents the entrance of elements into cells, the Nano particles which have less diameter than the pores of cell wall, therefore can easily cross the pores. Nano particles in the leaves's surface enter the plants through the stomata and or base of hairs, and are then transported to the different organs (18). Silica plays important role in the tolerance against salt stress [25]. Manganese toxicity [21], boron toxicity [11] and cadmium toxicity [23 , 20], via changing the activity of antioxidant enzymes. In the present study, silica was used as Nano particles with 14nm diameter (1.5 and 3 mM concentrations) to assay the effects of Nano particles of silica on some antioxidant enzymes such as catalase, peroxidase and superoxide dismutase changes, and the yield of broad bean plant.

MATERIAL AND METHODS

In order to assessment the effects of silica nano particles, on antioxidant activity of broad bean (*Vicia faba*) , the samples were grown in greenhouse. Before cultivation, the impact seeds were sterilized in 5% hypochlorite sodium solution. The seed then were washed up by demonized water. In each pot 2 seeds were cultivated. Solution containing 0 (as control) , 1.5 and 3 mM of nano particle of silica, were used for treating. The temperature of greenhouse was adjusted to 22±2 ^{0}C (at night) and 25±2C0 (at day). The relative humidity was 44 %. The samples were treated for 65 days and the fresh leaves of them kept in liquid nitrogen for enzyme assay.

Total protein:

[5] Method was used for total protein assay. 1 mL of Bradford solution was mixed with 100 µL of enzyme extract, and then the absorption was recorded in 595nM wave length. The protein concentration was expressed as Mg ml^{-1}

Catalase activity:

The activity of catalase was measured by (1) method. CAT activity was determined as the rate of disappearance of H_2O_2 at 240 nm, for 1 minute . Reaction mixture (3 ml) included 50mM potassium phosphate buffer (pH 7), and the activity was expressed as µmol min -1 per mg protein.

Peroxidase activity:

[14] Method was used to assay the activity of peroxidase. The mixture of 2mL acetate buffer (pH 4.8), 0.2 mL hydrogen peroxide 3% was used. The change in absorbance was determined at 590 nm (FW OD /min .g).

Superoxide dismutase activity:

The activity of superoxide dismutase was assayed by (10). Reaction mixture containing 50mM potassium phosphate buffer (pH 7.8), 1.3 µM riboflavin , 0.1 mM EDTA. 13 mM methionine, 63 µM NBT, 0.05 M sodium carbonate (pH 10.2) and enzyme extract , was used. The photoreduction of NBT was measured at 560 nm.

Statistic analysis

SPSS Ver16. Was used for comparing of the means using Duncan test at P<0/05, level of significance. The diagrams were plotted using Excel software.

RESULTS

Total protein

The result showed that the protein content in 1.5 mM treatment of Sodium silicate has no significant different to control sample. But this content in 3 mM treatment of nano silica was increased in range of 25% compared to control. This rage was about 7% in comparison with 1.5 mM of nano silica treatment.

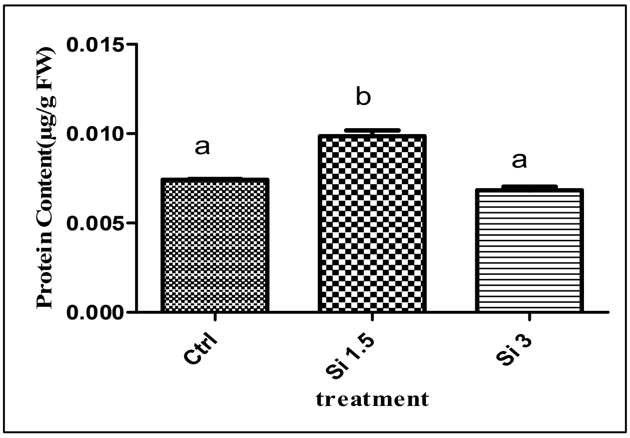

Fig 1: The effect of sodium silicateparticles on total protein content of broad bean leaves. Means ± SE and P ≤ 0.05. The letters show significance of differences.

Peroxidase activity:

The result showed that in the leaves of broad bean, 3mM treatments of sodium silicate, significantly increased the activity of peroxidase in range of 16 % compared to control samples respectively.Peroxidase enzyme activity was not significantly different between treatments sodium silicate.(Fig 2A).

Catalase activity:

The assessment of catalase activity indicated that in 3 mM treatment of sodium silicate the activity of this enzyme in leaves was significantly increased in a range of 15% compared to control samples. Asignificant decrease in catalase activityin49 percent of the sodium silicate was observed between treatments. Highest catalase activity of the sodium silicate treatment in a concentration3mM.(Fig 2B).

Superoxide dismutase activity:

The result showed that the activity of superoxide dismutase in leaves of broad bean plant, has highest level in 3mM treatment in comparison to control (71 % higher). Highest Superoxide dismutase activity of the sodium silicate treatment in a concentration1/5mM. However this difference was significant between 1.5 and 3 mM sodium silicate treatments (Fig 2C).

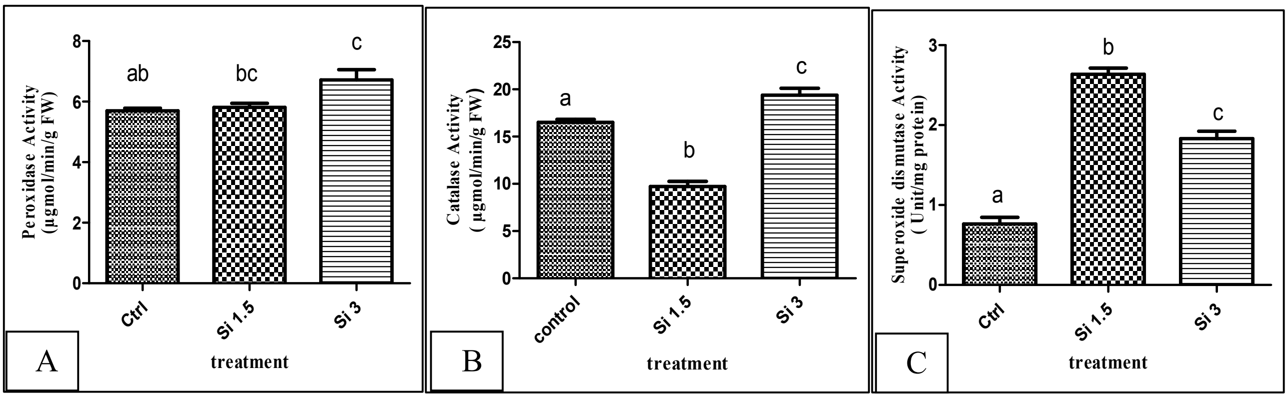

Fig 2: The effect of Sodium silicate particles on total protein content of broad bean leaves. Means ± SE and P ≤ 0.05. The letters show significance of differences. A) Peroxidase. B) Catalase . C) Superoxide dismutase.

DISCUSSION

The total protein content if 1.5mM treatment of sodium silicate showed no significant increasing compared to control sample. When plant's cell is under stress signaling pathway in corporation with calcium send signals to nucleus of cell. Due to this signaling, genes expression undergoes changes and because of increasing or decreasing of some genes, plant can resist against stress. Due to these genetic changes, content of special proteins changes [2]. [4] Showed that treatment of selenium can cause increasing of amino acid content, specially Asp in rice. The assessment of changes pattern of total protein content shows that under silica stress some new proteins can be generated, or the amount of some others can be increased or decreased [22]. Treatment of rice plant with silica brought about activity of catalase and Glycine betaine [4].Silica and nano particles of that, can act as a stress gen factor in leaves and as a result, the activity of antioxidant enzymes would be increased. These enzymes protect plants against toxicity and damages of reactive oxygen [24]. Catalase and ascorbate peroxidase can scavenge H_2O_2 in plant and therefore, the increasing of superoxide dismutase is also predictable. The activity of ascorbate peroxidase was increased in nano silica treatment. [17] Indicated that silica can compensate the effect of potassium shortage in soy bean. [13] Reported that treatment of rice with Sodium silicate increased the activity of catalase and ascorbate peroxidase.

CONCLUSION

The result of present study conclude that silica prevent oxidant damages via increasing of antioxidant enzymes activity and decreasing of free radicals.Silicon treatment with lower concentration of soluble protein increase the leaves. Due to the lack of information about the main mechanism of silica effects is yet unknown, more studies are needed to assay the uptake and transportation of nanoparticles in plants.

REFERENCES

1. Aebi, H., 1984 .Catalase in Vitro. Methods in Enzymology 105 : 121 – 126
2. Amini, F., A.A. Ehsanpour , Q.T Hong and J.Sh. Shin, 2007 .Protein Pattern Changes in Tomato Under in Vitro Salt Stress.Russian Journal of Plant Physiology, 54(4):464-471.
3. Bai, L., And F , Sui, 2006. Effect of Soil Drought Stress on Leaf of Maize.Pedosphere 16:326-332.

4. Biglari ,F., R. Hadad and A. Sotude, 2012 Assessment of Silica Treatments on Glysine Changes and Catalase, in Rice, in Drought Strees. The Second Conference of Plant Physiology of Iran.
5. Bradford, M.M. 1976 . A Rapid Sensitive Method for the Quantitation of Micro Program Quantities of Protein Utilizing the Principle of Protein- Dye Binding Anal Biochm., 72:248-254
6. Currie, H.A. And C . Perry, 2007 Silica In Plants: Biological, Biochemical and Chemical Studies. Annual Botany 100 (7): 1383-1389.
7. Elkahoui S., J. A. Hernandez, C. Abdelly, Ghrirr. and F. Limam, 2005 . Effects of Salt on Lipid.Peroxidation and Antioxidant Enzyme Activities of *CatharanthusRoseus*Suspension Cells.Plantscience. 168: 607-613.
8. Feierabend, J., C. Schaan and B. Hertwig,1992 . Photoinactivation of Catalase Occurs Under Both High and Low Temperature Stress Conditions and Accompanies Photoinhibition of Photosystem II. Plant Physiology. 100: 1554-1561.
9. Garnczarska, M.,2005. Response of the Ascorbate-Glutathione Cycle to Reaeration Following Hypoxia in Lupine Roots.Plant Physiology and Biochemistry. 43(6): 583-590.
10. Giannopolitis, C.N. and S.K. Ries. 1977. Superoxide Dismutase Occurrence in Higher Plants . Plant Physiology 59 : 309 – 314
11. Gunes, A., A. Inal and E.G. Bagic, 2007. Silicon Mediated Changes of Some Physiological and Enzymatic Parameters Symptomatic for Oxidative Steress in Spinach and Tomato Grown in Sodic – B Toxic Soil.Plant Soil 290: 103- 114
12. Kakkar R. K. And V. K. Sawhney,2002. Polyamine Research in Plants-A Changing Perspective.Physio.Planta. 116: 281-292.
13. Kiani .Z., A .Abdolzadeh and H. Sadeghipour ,2012. Assessment of Silica Treatment on Shortage of Iron in Rice.Iranian Biology Magazine. Volume 4 Pages 61-71.
14. Koroi , S.A.A ., 1989 , Gelekektro phersstische and spectral photo metrischoe under uchungen zomeinfiuss der temperature auf straktur and aktritatder amylas and peroxidise isoenzyme , Physiology Vegetatio , 20 : 15-23.
15. Liang, Y., W. Sun, Y. Zhu and P.Christie, 2007 Mechanisms of Silicon-Mediated Alleviation of Abiotic Stresses in Higher Plants- A Review. Environmental *Pollution* 147: 422-428.
16. Ma, J. F. And N. Yamaji ,2006. Silicon Uptake and Accumulation in Higher Plants. Plant Science 11: 392-397.
17. Miao, B. H ., X. G. Han And W. H. Zhang, 2010. The Ameliorative Effect of Silicon on Soybean Seedlings Grown in Potassium-Deficient Medium. Annals of Botany 105:967-973.
18. Nair,R., S.H.Varghese, B.G.Nari,T.Maekawa,Y.Yoshida and D. Sakthi Kumar.2010.review Nanoparticulate material delivery to plants.plant Sci.179 : 154-163.
19. Richmond, K. E.and M. Sussman, 2003. The Non-Essential Beneficial Plant Nutrient. Current Opinion in Plant Biology 6: 268-272
20. Shi, G., Q. Cai, C. Liu AndL.Wu, 2010 . Silicon Alleviates Cadmium Toxicity in Peanut Plants in Relation to Cadmium Distribution and Stimulation of Antioxidative Enzymes. Plant Growth Regulation 61: 45-52.
21. Shi , X.H., C.C . Zhang, H .Wang and F.S. Zhang ,2005 .Effect Of Si on The Distribution of Cd in Rice Seedling . Plant and Soil 273:53- 60.
22. Torabi,F.andA.Majd, 2013. Interaction of Silica Sodium and Chloride Sodium on Seed Germination and Ontogeny of oxtongue . 151
23. Vaculik, M., A .Lux, M. Luxova, E. Tanimoto and I .Lichtscheidl, 2009. Silicon Mitigates Cadmium Inhibitory Effects in Young Maize Plants. Environmental and Experimental Botany 67: 52-58.
24. Van Breusegem, F., L. Slooten and J.M. Stassart, 1999. Overproduction of *ArabidopsisThaliana* Fe SOD Confers Oxidative Steress Tolerance to Transgenic Maize. Plant Cell Physiol 40:515-523
25. Zhu, J., 2002 .Regulation Homeostasis Under Salt Steress. Curent Opinion in Cell Biology 141-145.

CITATION OF THIS ARTICLE

Ghffar R,Sadigheh A, Golnaz T ,Ahmad M,Fahimeh S.The study of Sodium silicate effects on the total protein content, and the activities of catalase, Peroxidase and Superoxide Dismutase of *Vicia faba* L. Bull. Env. Pharmacol. Life Sci., Vol 3 [Spl Issue V] 2014: 243-246